(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 June 2002 (20.06.2002)

PCT

(10) International Publication Number
WO 02/48379 A1

(51) International Patent Classification[7]: C12N 15/85

(21) International Application Number: PCT/KR01/02178

(22) International Filing Date:
14 December 2001 (14.12.2001)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
2000/77279 15 December 2000 (15.12.2000) KR
2001/79227 14 December 2001 (14.12.2001) KR

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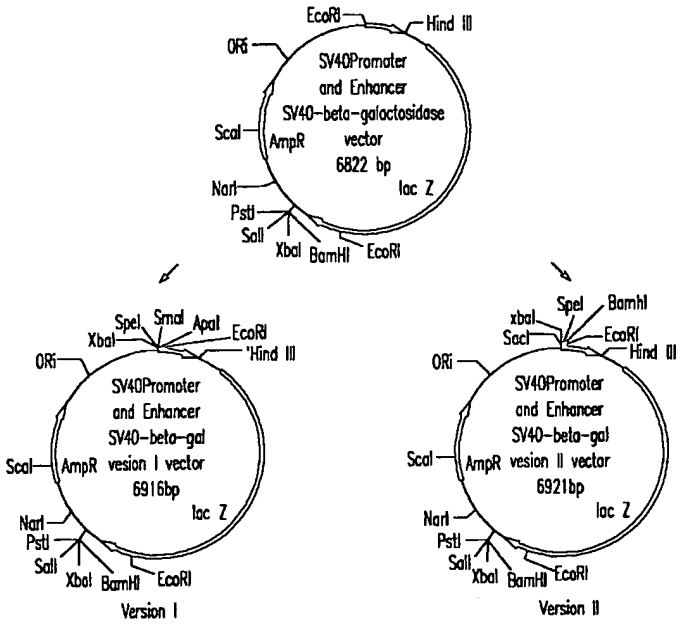
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK,

[Continued on next page]

(54) Title: EXPRESSION VECTOR FOR ANIMAL CELL CONTAINING NUCLEAR MATRIX ATTACHMENT REGION FO INTERFERON BETA



(57) Abstract: The present invention relates to mammalian expression vectors including nuclear matrix attachment region of human interferon β, and more particularly to pPGM-1, pPGM-2 and pPGM-3 including nuclear matrix attachment region of interferon β gene. Those expression vectors confer position independent expression of the introduced foreign gene, thus increasing the frequency of colonies which efficiently express the recombinant protein.

WO 02/48379 A1

SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *entirely in electronic form (except for this front page) and available upon request from the International Bureau*

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EXPRESSION VECTOR FOR ANIMAL CELL CONTAINING NUCLEAR MATRIX ATTACHMENT REGION OF INTERFERON BETA

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to mammalian expression vectors including a nuclear matrix attachment region of interferon β gene, and more particularly to pPGM-1, pPGM-2, and pPGM-3.

(b) Description of the Related Art

Various expression systems including microorganisms, plants, yeasts, insect cells, mammalian cells, etc. have been used to express and obtain target proteins in large quantities to apply to medical and industrial uses. Microorganisms are the easiest systems to use, and microorganism expression systems suitable for various applications have been developed and are commonly used.

However, microorganism expression systems have some limitations. The most serious limitation is that since protein expression and modification mechanisms (glycosylation, phosphorylation, amidation) of microorganisms differ from those of mammalian cells, even if the same gene is expressed in a microorganism system, the structure or characteristics of expressed proteins is not completely identical to the original protein. Therefore, production of recombinant proteins using microorganism expression

systems frequently expresses proteins that are inactivated because modification does not occur after synthesis, or that partially differ in modification or structure even if they are not significantly different in function. In addition, the recombinant protein production process using microorganism expression systems should be accompanied by additional contaminant removal due to contamination of microorganisms, contamination of microorganism endotoxin, etc.

Meanwhile, mammalian expression systems, although they are the most suitable systems for expressing mammalian proteins, have not been easily industrialized because recombinant protein expression efficiency is low and thus the unit cost of production is high, and the mammalian cell handling process is difficult. Presently used industrial mammalian cell lines include CHO (Chinese Hamster Ovary), BHK (Baby Hamster Kidney), myeloma, etc., and an expression vector including the gene of interest is transfected into the mammalian cell line to express aimed foreign proteins.

Mammalian cells maintain various protein modification mechanisms including glycosylation, and protein obtainment and purification processes are easier when proteins are secreted to a culture medium. Most mammalian cells require complex additives such as serum protein, etc. in the culture process, while CHO cells can be cultured in a medium without serum and protein, rendering it the most suitable host for expression of recombinant proteins. In addition, characteristics of CHO cells are well known due to their having been used in many studies, and they have

advantages of a high growth rate and that mass suspension culture is possible.

Generally, in order to express a transgene in a mammalian cell, a vector having a selection marker and a transgene are simultaneously transfected. Transfected cells are cultured and selected in a selection medium. However, expression frequency thereof is very low. One of the reasons is that these transgenes should be integrated in chromosomes of a host cell in mammalian cells contrary to the microorganism system. Additionally, even if stable transfectants are selected, the expression amount is difficult to predict. This is because gene integration positions differ according to cells, and expression aspects differ according to integration positions. Therefore, the copy number of transgenes in mammalian cells and the gene expression amount do not have an explicit correlation therebetween (Grindley et al., 1987, Trends Genet. 3, 16-22; Kucherlapati et al., 1984, *Crit. Rev. Biochem.* 16, 349-381; Palmiter et al., 1986, *Annu. Rev. Genet.* 20, 465-400). Gene expression in mammalian cells is mostly repressed by a nucleic acid base near the integration position, and thus stably integrated transgenes would often be expressed in a very low level (Eissenberg et al., 1991, Trends Genet. 7., 335-340; Palmiter et al., 1986, *Annu. Rev. Genet.* 20, 465-499).

Usability of nucleic acid factors for protecting transgene expression from position effects has been reported in many systems. As the nucleic acid factors, an insulator factor and a nuclear matrix attachment region

(MAR) or scaffold attachment region (SAR), etc. can be used. Although operation mechanisms thereof have not been clarified, when included in transgene constructs, they induce position independent gene expression and the expression amount is determined by the copy number of gene (McKnight, R.A. et al., 1992, *Proc. Natl. Acad.* US. 89, 6943-6947).

Kalos et al. have combined the MAR factor of the human apolipoprotein B gene with a minimal promoter transgene construct and induced gene expression in mammalian cells to increase expression of the transcript by about 200 times (Kalos et al., 1995, *Mol. Cell. Biol.* 15, 198-207). Similarly, it has been reported that the MAR factor of the chicken lysozyme A gene and the SAR factor of human interferon β , etc. confer position-independent transgene expression in vertebrates (Eissenberg et al., 1991, Trends Genet. 7, 335-340; Klehr et al., 1991, *Biochemistry* 30, 1264-1270). However, no attempt to apply the MAR/SAR factor to substantially increase protein production in CHO cell lines has been reported, nor has industrial profit been identified.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a vector which confers increased foreign protein expression efficiency in mammalian cells.

It is another object of the present invention to provide a vector for position independent expression of foreign proteins.

It is still another object of the present invention to provide a vector comprising a nuclear matrix attachment region of interferon β gene for increasing the frequency of foreign protein expression and the amount of foreign protein expressed in mammalian cells.

It is further object of the present invention to provide a vector comprising multiple cloning sites to facilitate the cloning of transgenes.

It is still further object of the present invention to provide a vector capable of expressing foreign proteins in mammalian cells.

In order to achieve these objects, the present invention provides mammalian expression vectors comprising a nuclear matrix attachment region of interferon β gene, a promoter, and a transcription terminator.

The present invention also provides mammalian cells transfected with the expression vectors in which target genes are introduced.

The present invention also provides a method for production of proteins comprising the steps of introducing transgenes into mammalian expression vectors and introducing the vector into mammalian cells to express proteins from the transgenes.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a structure of a vector constructed by modifying a pSV-β -gal vector in order to construct the expression vector of the present invention.

Fig. 2 shows the β -gal staining method which demonstrates the

increase in the frequency of positive cells by nuclear matrix attachment region of interferon β gene.

Fig. 3 shows frequency of positive cell lines that express β -gal when amplifying introduced genes by adding MTX

Fig. 4 shows β -gal expression frequency and expression amount in a G418 selection medium.

Fig. 5 shows β -gal expression frequency and expression amount in a DHFR selection medium.

Fig. 6 shows β -gal expression frequency and expression amount in the presence of a CMV promoter.

Fig. 7 shows the structure of the pPGM-1 expression vector of the present invention.

Fig. 8 shows the map of the pPGM-2 expression vector of the present invention and a base sequence of multiple cloning sites.

Fig. 9 shows the map of the pPGM-3 expression vector of the present invention.

Fig. 10 shows results of analyzing expression titer of a human growth hormone expression cell line prepared using pPGM-1, by Western Blot.

Fig. 11 shows results of analyzing expression titer of an interferon β expression cell line prepared using pPGM-1, by Western Blot.

DETAILED DESCRIPTION AND THE PREFERRED EMBODIMENTS

The present inventors invented optimum expression vectors for overcoming position-specific expression inhibition effect (hereinafter referred to as "position effect") and improving gene expression amounts in transgene expression in mammalian expression systems.

The mammalian expression vector of the present invention further added a useful base sequence to the existing expression vectors. The useful base sequence protects transgene expression from position effects of the host cell chromosome, and increases transgene expression in CHO (Chinese hamster ovary) or BHK (Baby hamster kidney) cells, etc., commonly used as commercial mammalian cells. The useful base sequence is preferably the nuclear matrix attachment region (MAR) or saffold attachment region (SAR).

The MAR/SAR factor is preferably selected from a group consisting of chicken lysozyme 5' MAR (Phi-Van, L. and Stratling, W.H., 1996, *Biochemistry* 35, 10735-10742, Gene bank #: X98408), chicken phi α globin 5'MAR (Kraevskii, V.A. et al., 1992, *Mol. Biol.* 26, 672-678, Gene bank #: X64113), CHO DHFR intron MAR (Kas, E. and Chasin, L.A., 1987, *J. Mol. Biol.* 198, 677-692, Gene bank #: X06654), human HRRT intron MAR (Sykes, R.C. et al., 1988, *Mol. Gen. Genet.* 212, 301-309, Gene bank #: X07690), human CSP-B gene flanking SAR (Hanson, R.D. and Ley, T.J., Gene bank #: M62716), and human interferon β gene flanking SAR (Mielke, C. et al., 1990, *Biochemistry* 29, 7475-7485, Gene bank #:

M83137).

Influences of the MAR/SAR factor on transgene expression were analyzed by integrating 6 kinds of MAR/SAR factors into pSV-β -gal/ver I or pSV-β -gal/ver II and examining expression aspects of β -gal genes.

Six kinds of MAR/SAR factors were respectively integrated into upstream of a promoter of pSV-β -gal/ver I or pSV-β -gal/ver II to complete a test vector. The test vector was introduced to CHO DG44 cells to prepare a transfected cell line, and β -gal expression frequency and expression amount were measured.

The recombinant vector pSBβ /I including interferon β MAR factor was compared with pSV-β -gal as a control. The number of colonies increased by about 3 times in a G418 medium, and frequency of positive cell lines expressing β -gal increased to 71% compared to 35% of the control, thus the number of positive cell lines increased by 6 times.

In addition, when pSVβ /I transfected cell lines were selected in a DHFR selection medium, the number of positive colonies that were produced in initial selection medium not including nucleoside increased by 10 times. When MTX was added to a culture medium to induce transgene amplification, the β -gal positive colony rate of the pSVβ /I transfected cell line was 90% or more of total colonies, and the number of positive cells increased to about 7 times as much as the control PSV-β -gal (Fig. 3).

Accordingly, the interferon β MAR factor of the present invention

increases expression efficiency of transgenes and selection marker genes to largely enhance growth of transfected cell lines in selection medium, and shortens periods required for selecting transfected cell lines in selection mediums.

The present invention also provides mammalian expression vectors including a nuclear matrix attachment region of interferon β at a upstream of promoter 5'. The mammalian expression vectors are preferably pPGM-1, pPGM-2, and pPGM-3. The promoter is preferably selected from a group consisting of SV40 promoter, CMV (cytomegalovirus) promoter, and MMTV (Mouse Mammary Tumor Virus) promoter.

The pPGM-1 vector contains a 5409bp including a nuclear matrix attachment region of the human interferon β gene, a SV40 virus promoter, and multiple cloning sites (MCS), and it can express various genes by integrating transgenes in multiple cloning sites. Multiple cloning sites include *Hind*III, *Nhe*I, *Not*I, and *Xho*I restriction sites. The base sequence of the pPGM-1 vector is listed as SEQ ID NO: 1 in the sequence listing program, and major factors included in the vector and their positions are shown in Table 1 and Fig. 7. The pPGM-1 vector was deposited with the Korean Culture Collection of Microorganisms under deposition no. KCCM 10232.

Table 1

Sequence No.	Functions
1-413	Initial promoter and enhancer of SV40 virus
414-448	Multiple cloning sites (MCS)

449-584	Small T antigen of SV40 virus
1194-2054	β -lactamase: Amp^R gene
3225-5397	Nuclear matrix attachment region of interferon β gene

The pPGM-2 vector, which modifies multiple cloning sites of the pPGM-1 vector, includes a nuclear matrix attachment region of the interferon β gene, a SV40 promoter, and multiple cloning sites. The base sequence of the pPGM-2 is listed as SEQ ID NO: 2, and the structure of the vector is shown in Fig. 8 and Table 2. The multiple cloning sites of the pPGM-2 vector include *Nhe*I, *Pme*I, *Af*III, *BamH*I, *BstX*I, *EcoR*V, *Not*I, *Xho*I, *Xba*I, *Apa*I, *Pme*I, and *Mlu*I restriction sites. The pPGM-2 vector was deposited with the Korean Culture Collection of Microorganisms under deposition no. KCCM 10338.

Table 2

Sequence No.	Functions
1-413	SV40 promoter
426-445	T7 promoter
446-579	Multiple cloning sites (MCS)
1331-2191	β -LACTAMASE: Amp^R gene
3361-5534	Nuclear Matrix Attachment Region of interferon β gene

The pPGM-3 vector which substitutes the SV40 promoter of the pPGM-1 vector with the CMV (Cytomegalovirus) derived promoter consists of 5601 bp. The base sequence thereof was shown as SEQ ID NO:. 3, and the structure is shown in Fig. 9 and Table 3. The pPGM-3 vector was deposited with the Korean Culture Collection of Microorganisms under KCCM 10339.

Table 3

Sequence No.	Functions
1-611	SV40 promoter
612-640	Multiple cloning sites (MCS)
1396-2246	β -lactamase:Amp^R gene
3417-5589	Nuclear Matrix Attachment Region of interferon β gene

The pPGM-1 vector, pPGM-2 vector, and pPGM-3 vector of the present invention are superior to common vectors in number of positive cells expressing transgenes, frequency, and expression amount. Therefore, the pPGM-1 vector, pPGM-2 vector, and pPGM-3 vector of the present invention can be used for expressing and producing transgenes in mammalian cells, and they can produce recombinant proteins such as enzyme and cytokine.

In addition, the present invention introduces a transgene into a vector including a nuclear matrix region of the interferon β gene, and introduces it into a mammalian cell to provide a transfected cell line. The transfected cell line is selected by culturing in a medium to which a selection marker or MTX is added. A transgene is a gene encoding all kinds of proteins capable of being expressed as recombinant proteins. Representative examples include insulin, cytokine (interleukin, tumor necrosis factor, interferon, colony stimulation factor, chemokine, etc.), erythropoietin, etc. The transfected cell line can be produced by a common method.

In addition, the present invention provides a method of producing proteins comprising : introducing a transgene into a vector including a

nuclear matrix attachment region of the interferon β gene, and introducing the vector into a mammalian cell to express proteins from a transgene. The vector is preferably selected from a group consisting of pPGM-1 (KCCM 10232), pPGM-2 (KCCM 10338), and pPGM-3 (KCCM 10339). The mammalian cell can be any mammalian-derived cell, and it is preferably CHO (Chinese hamster ovary).

As described above, the pPGM-1 vector, pPGM-2 vector, and pPGM-3 vector of the present invention improve repression of transgene expression caused by surrounding bases and increase expression. In addition, the expression vector of the present invention can be effectively applied to mass production of industrially useful proteins, and it can produce recombinant proteins having the same structure and functions as original proteins of higher animals.

The present invention will be explained in further detail with reference to the following Examples and Comparative Examples. However, these are to illustrate the present invention and the present invention is not limited to them.

Example 1: Cloning of MAR or SAR factor

Genomic DNA was isolated from human G-2 cells using a Wizard Genomic DNA purification kit (Promega, U.S.A.). 10 μg of DNA were cut with *Cla*I, *Sma*I, *Xba*I, and *Xho*I restriction enzymes to use in the PCR process. DNA to be used in PCR was separated and purified from a CHO chicken cell line and a chicken embryo by a similar method.

200 ng of the isolated genomic nucleic acid was used as a template, and 25 pmole of each primer, 0.5 mM of dNTP, and ExTaq polymerase (Takara Shuzo Co., Japan) were added to carry out PCR. The base sequence of primer used for each MAR/SAR factor and size of DNA fragment obtained by PCR are as shown in Table 4. PCR was carried out using a GeneAmp PCR system 9600 (Perkin – Elmer Corp. U.S.A.), and PCR conditions are as shown in Table 5.

Table 4

MAR/SAR factor	Primer	SEQ ID NO:	Size
Chicken lysozyme 5'MAR	5'-GGA TCC ATA ATA TAA CTG TA-3'	4	1668 bp
	5'-AAG CTT AAA AGA TTG AAG CA-3'	5	
Chicken phi α globin 5'MAR	5'-AAG CTT TTA ACC AAC AAA AA-3'	6	619 bp
	5'-CTG CAG ACC TAA CCT GTC AC-3'	7	
CHO DHFR intron MAR	5'-TAT ACG TGA ATA GTT TTT CT-3'	8	549 bp
	5'-GAG TTG GAA CTG AGA AGT TC-3'	9	
Human HPRT intron MAR	5'-AAG CTT GGT CAA GA TGG TG-3'	10	580 bp
	5'-GCT GGG CGT GGT GGT GCC TG-3'	11	
Human CSP-8 gene SAR	5'-GGA TCC GAT TCT CCT TGA TG-3'	12	1233 bp
	5'-GAA TTC AAA CAA CTC AAT AG-3'	13	
Human interferon β SAR	5'-GAA TTC AGC AAG GTC GCC AC-3'	14	2174 bp
	5'-TTG TAT CAA CTT TCT ACA AT-3'	15	

Table 5

Step	Conditions	Cycle
1	94℃, 2 min	1
2	94℃, 40 sec; 65℃, 40 sec; 72℃, 40 sec	2-31
3	72℃, 10 min	32

Fragments of chicken lysozyme 5' MAR (Gene bank #: X98408, hereinafter referred to as "lyso MAR"), chicken phi α globin 5' MAR (Gene

bank #: X64113, hereinafter referred to as "phi-a MAR"), CHO DHFR intron MAR (Gene bank #: X06654, hereinafter referred to as "DHFR MAR"), human HPRT intron MAR (Gene bank #: X07690, hereinafter referred to as "HPRT MAR"), human CSP-B gene flaking SAR (Gene bank #: M62716, hereinafter referred to as "CSP-B MAR"), and human interferon β gene flanking SAR (Gene bank #: M83137, hereinafter referred to as "interferon β MAR") were separated by the above-explained method.

The PCR product was subcloned in pT7blue (R) (Novagene, U.S.A.) or a pCR 2.1 (Invitrogen, U.S.A.) vector. HPRT MAR, DHFR MAR, and lyso MAR were subcloned in a pT7blue (R) vector, and phi-a MAR, CSP-B MAR, and interferon β MAR were subcloned in PCR 2.1.

Example 2: Construction of pSV-β -gal/ver I and pSV-β -gal/ver II vectors

In order to effectively clone multiple MAR/SAR factors subcloned in the pT7blue (R) or pCR 2.1 vector at upstream of a SV40 promoter of a pSV-β -gal (hereinafter referred to as 'pSVβ ') vector, modified pSV-β -gal/ver I and PSV-β -gal/ver II vectors were constructed (Fig. 1).

(1) Construction of pSV-β -gal/ver I

PCR was carried out with pSVβ as a template with primers of SEQ ID NO:. 16 and SEQ ID NO: 17 to obtain a fragment including the SV40 promoter. The fragment was cut with *Spe*I and *Hind*III enzymes, and a

443 bp of DNA fragment including SV40 was purified with a Geneclean III kit (Bio 101, U.S.A). It was subcloned in a opened pBluescript SK (+) vector (Stratagene, U.S.A.) with *Spe*I and *Hind*III enzymes to construct pBS/SV40 I.

The pBS/SV40 I was digested with *Sca*I and *Hind*III enzymes to separate a 1240 bp DNA fragment, and it was subcloned in pSVβ treated with the same enzymes to complete a pSV-β -gal/ver vector.

(2) Construction of pSB-β -gal/ver II vector

A pSVβ was cut with *EcoR*I and *Hind*III to obtain a 420 bp fragment by the above-explained method, and the fragment was inserted into a pBluescript SK (+) vector opened with the same enzymes to construct a pSV-β -gal/ver II vector.

Example 3: Construction of a vector including MAR factor and β -gal gene

MAR factors were separated from pT7blue(R)/HPRT MAR, bT7blue(R)/DHFR MAR, pT7blue(R)/lyso MAR, pCR 2.1/phi-a MAR, pCR 2.1/CSP-B MAR, and pCR 2.1/interferon β MAR, and cloned in pSVβ /I or pSVβ /II.

In a pSVβ /I, phi-a MAR and HPRT MAR (human HPRT intron MAR) were subcloned using *Spe*I/*Sma*I, and interferon β MAR and lyso MAR were subcloned using *Apa*I/*Spe*I. Constructed vectors were pSV-β -

gal/phi-a MAR , pSV-β -gal/HPRT MAR, pSV-β -gal/interferon β MAR (hereinafter referred to as 'pSVβ /I'), and pSV-β -gal/lyso MAR.

Additionally, DHFR MAR and CSP-B MAR were subcloned in pSV-β -gal/ver II at *BamHI*/*Xba* I restriction site. Constructed vectors were pSV-β -gal/DHFR MAR and pSV-β -gal/CSP-B MAR respectively.

Example 4: Construction of pCMVβ and pCMVβ /I

A *SpeI*-*XhoI* fragment including a MAR factor was deleted from a pSVβ /I vector and the vector was self-ligated. After cleavage by *ApaI* and *Hind*III, a CMV promoter fragment was inserted to construct a pCMVβ vector. The CMV promoter was amplified by PCR with primers of SEQ ID NO: 18 and SEQ ID NO:19 in a pcDNA3.1 vector. The sequences of SEQ ID NO: 18 and SEQ ID NO:19 include a *ApaI* or *Hind*III restriction site.

Also, a pSVβ I vector was digested with *ApaI* and *Hind*III restriction enzymes and a CMV promoter fragment was integrated therein to construct a pCMVβ /I vector.

Namely, pCMVβ is a vector without the MAR factor, and pCMVβ /I is a vector having the MAR factor.

Example 5: Measurement of gene expression of pSVβ and pSVβ /I

(1) Transfection

A CHO cell line DG44 deficient of the DHFR gene was cultured in a MEM-α medium (α -Minimum Essential Medium, Gibco BRL) to which 10% serum (fetal bovine serum) was added. 2 X 10^5 cells were inoculated on a 60 mm plate together with 3 ml of medium and cultured in 5% CO_2 incubator at 37℃ overnight, until transfection occurred.

Transfection was carried out by a liposome method. A pSV2Neo or pDCH1P vector having selection maker gene was co-transfected with each test vector in a mole ratio of 100:1, and a pSVβ vector was used as a control to perform the same experiment. 2 μg of each vector were mixed with lipid surfactant, DOSPER (BOEHRINGER Mannheim, German) and serum-free MEM-α medium and reacted at room temperature for 45 minutes, it was added to a rinsed cell together with a medium to culture for 5 hours, and then the reaction liquid was removed. Culture was carried out in MEM-α medium including 10% FBS and G418 (500 μg/ml) for 2 weeks in order to select the transfected cell line using a Neo gene, and culture was carried out in MEM-α medium not including a nucleoside for 2 weeks in order to select a DHFR gene, and β -gal expression was analyzed.

(2) Measurement of frequency of β -gal expression positive cell line

Positive cells expressing β -gal were selected.

Cells cultured on a 100 mm plate were treated with a fixing agent (2% formaldehyde and 0.2% glutaraldehyde) at 4 ℃ for 10 minutes. They

were washed with 1X PBS twice, and X-gal (1 mg/ml) was added to react them at 37 ℃.

A β -gal expression cell can be easily identified because it decomposes X-gal and shows a blue color. Positive cells were separated with a trypsin-EDTA solution, and the number of cells was measured with a hematocytometer.

Fig. 2 is a photo of cells treated with X-gal after being transfected with pSVβ or pSVβ /I in order to identify β -gal expression cells. (a) shows cells transfected with pSVβ , and (b) shows cells transfected with pSVβ /I. A positive cell line showing a blue color of PSVβ /I transfected cell line is remarkable in the upper plate photo.

After transfection, the transfected cell line was selected using a Neo gene or a DHFR gene, and the number of positive cells expressing β -gal were measured.

Table 6 shows the number of β -gal positive cells of DG44/PSVβ or DG44/PSVβ /I selected using the Neo selection factor. Table 7 shows the number of β -gal positive cells of DG44/pSVβ or DG44/pSVβ /I selected using the DHFR selection factor.

Table 6

	Total cell number	Positive Cell number	Negative Cell number	Positive Cell Frequency (%)
DG44/pSVβ	222	145	77	34.68
DG44/pSVβ /I	659	191	468	71.02

Table 7

	Total Cell number	Positive Cell number	Negative Cell number	Positive Cell Frequency (%)
DG44/pSVβ	120	105	15	12.50
DG44/pSVβ /I	350	200	150	42.86

The total number of colonies and frequency of positive colonies increased, and the number of positive colonies remarkably increased, when the transfected cell lines were selected by culturing in selection maker existing media.

In addition, the transfected cell line (DG44/pSVβ , DG44/pSBβ /I) was adapted to a MTX-added medium to induce gene amplification. The β -gal expression frequency of the transfected cell line adapted to MTX 10 nM and 50 nM was measured. Fig. 3 is a graph measuring activity of the β -gal of MTX-adapted transfected cell line. 90% or more of the DG44/PSVβ /I transfected cell line selected in MTX expressed β -gal, and produced 7 times as many positive colonies as the control (pSVβ).

Accordingly, the interferon β MAR factor further enhanced expression activity of the SV40 promoter to increase frequency of positive cell lines by 6-10 times. A common transfected cell line preparation process requires that transfected cell lines should be selected from a selection medium for a very long period. However, it is expected that the MAR factor will sharply shorten the period of time required for development of transfected cell lines. In addition, the MAR factor affects expression of

selected genes as well as target genes to largely enhance growth of recombinant cell lines in the selection medium.

(3) β -gal activities of DG44/pSVβ and DG44/pSVβ /I

β -gal activity of the same cell as used to measure frequency of the β -gal expression positive cell line was measured.

A transfected cell line was cultured for 48 hours and washed with 1X PBS twice. 1 ml of STE (0.1M NaCl, 10 mM Tris-Cl (pH 8.0) and 1 mM EDTA (pH 8.0) solution were added thereto and it was put on ice. Cells were scraped with a scraper, moved to an effendorf tube, centrifuged at 4 ℃ at 14000 rpm for 40 seconds to remove supernatant, and 100 ㎕ of 0.25 M Tris-Cl (pH 7.5) was added to mix with the cells. A process for freezing under liquid nitrogen and immediately melting at 37 ℃ was repeated 5 times. The pulverized cells were centrifuged at 4 ℃ at 12000 rpm for 10 minutes and supernatant was moved to a new tube to analyze it.

30 ㎕ of cell extracts, 3 ㎕ of 100 X magnesium solution (0.1 M $MgCl_2$, 4.5 M β -mercaptoethanol), 66 ㎕ of 1 X ONPG (4 ml/ml in 0.1 M sodium phosphate (pH 7.5)), and 201 ㎕ of 0.1 M sodium phosphate (pH 7.5) were mixed well, and reacted at 37 ℃ until the color changed to yellow. After adding 500 ㎕ of 1M Na_2CO_3, absorbance was measured at 420 nm.

The protein amount in solution was measured by the bicinchoninic acid (BCA) method (Smith et al., 1985, Anal. Biochem. 150, 76-85).

Fig. 4 is a graph measuring β -gal activity of DG44/pSVβ or DG44/pSVβ /I selected with the Neo selection maker. Fig. 5 is a graph measuring β -gal activity of DG44/pSVβ or DG44/pSVβ /I selected with the DHFR selection maker.

When the MAR factor existed in the expression vector, β -gal activities increased 7.5 times (Fig. 4) and 32 times (Fig. 5), respectively. This indicates that, considering the frequency of positive cells, expressed β -gal protein activity per positive cell substantially increased.

Example 6: Measurement of β -gal expression of pCMVβ and pCMVβ /I

Each of pCMBβ and pCMVβ /I was transfected into DG44 by the same method as in Example 5, and number of β -gal positive cell lines and activities were measured.

Fig. 6 is a graph showing β -gal activities of DG44/pCMVβ and DG44/pCMVβ /I, and Table 8 shows the number of β -gal positive cells of DG44/pCMVβ and DG44/pCMVβ /I.

Table 8

	Total Cell number	Positive Cell number	Negative Cell number	Positive Cell Frequency (%)
DG44/pCMVβ	180	147	33	18.33
DG44/pCMVβ /I	326	79	247	75.77

The MAR factor increased frequency of positive cell lines from

18.33% to 75.77% even under a CMV promoter, and it increased the total number of colonies by about 1.8 times and the number of positive colonies by about 7.5 times. Therefore, it is suggested that the effects of interferon β MAR factor are not limited to the SV40 promoter, and that the MAR factor of the present invention can be used in various promoter including SV40, CMV promoter, and other promoters.

Example 7: Construction pPGM-1 vector

A β -gal site was deleted from a pSVβ /I vector and multiple cloning sites (MCS) were insertedtroduced to construct a pPGM-1 vector with the genetic map of Fig. 7.

A *Hind*III – *BamH*I fragment including a β -gal gene was removed from a pSVβ /I vector, and 160 bp of a *Hind*III/*BamH*I fragment of pMSG (KCCM 10202) was inserted. The pPGM-1 vector was deposited with the Korean Culture Collection of Microorganisms and assigned deposit No. KCCM 10232.

Example 8: Construction of pPGM-2 vector

A pPGM-1 vector was inserted with multiple cloning sites to construct a pPGM-2 vector.

A *Hind*III – *BamH*I fragment of a pSVβ vector was substituted with a pMSG (KCCM 10202) derived corresponding fragment, and the vector was opened with an EcoRI enzyme to integrate an interferon MAR factor. It was treated with NheI and XhoI enzymes and a fragment including

multiple cloning sites and a T7 promoter was ligated. The fragment was prepared using a PCR primer (Sequence No. 20 and Sequence No. 21) comprising *Avr*II and *Sal*I restriction site.

Fig. 8 shows the map of the pPGM-2 vector and base sequence of multiple cloning sites, and the vector was deposited with the Korean Culture Collection of Microorganisms and assigned deposit No. KCCM 10338.

Example 9: Construction of pPGM-3 vector

A *HindIII – BamHI* fragment of pSVβ -1 vector was substituted with a pMSG (KCCM 10202) derived corresponding fragment. The vector was digested with *EcoR*I and *Nhe*I to delete a promoter and substitute it with a CMV promoter, and it was opened with an *EcoR*I enzyme to ligate an interferon MAR factor. The structure of the constructed pPGM-3 vector is shown in Fig. 9, and its deposit No. is KCCM 10339.

Example 10: Production of recombinant protein using pPGM-1 vector

The pPGM-1 vector was digested with *Nhe*I and *Xho*I enzymes and integrated genes were ligated. The genes were cDNA encoding human growth hormone (Gene Bank #: E01424) or human interferon β (Gene Bank #: V00534).

The constructed expression vector was transfected into CHO DG44 cells and a high expression transfected cell line was selected while increasing the concentration of MTX added to a medium. Expression titer was measured by Western Blot, as shown in Figs. 10 and 11.

1.08 X 10^5 cells were put in a 12-well plate and cultured for 48 hours, and the medium was pooledto carry out Western Blot. Lane 1 is 25 ng of positive control protein, and lanes 2 – 5 are culture solutions derived from different colonies selected. The expression rate for human growth hormones was measured at about 20 μg/ml/day, and the expression rate for interferon β was approximately 15 μg/ml/day.

WHAT IS CLAIMED IS:

1. A mammalian expression vector comprising:

(a) a nuclear matrix attachment region of interferon β gene;

(b) a promoter; and

(c) a transcription terminator.

2. The mammalian expression vector according to Claim 1, wherein the promoter is selected from a group consisting of SV40 promoter, CMV (cytomegalovirus) promoter, and MMTV (mouse mammary tumor virus) promoter.

3. The mammalian expression vector according to Claim 1, wherein the mammalian expression vector is pPGM-1 KCCM 10232.

4. The mammalian expression vector according to Claim 1, wherein the mammalian expression vector is pPGM-2 KCCM 10338.

5. The mammalian expression vector according to Claim 1, wherein the mammalian expression vector is pPGM-3 KCCM 10339.

6. A cell transfected with a mammalian expression vector of Claim 1 in which target gene was inserted.

7. The cell according to Claim 6, wherein the mammalian expression vector is selected from a group consisting of pPGM-1 (KCCM 10232), pPGM-2 (KCCM 10338), and pPGM-3 (KCCM 10339).

8. The cell according to Claim 6, wherein the cell is a mammalian cell or a cell derived from a mammalian organism.

9. The cell according to Claim 6, wherein the cell is derived from

CHO (Chinese hamster ovary) cell line.

10. A method of producing recombinant protein comprising:

introducing a transgene into the mammalian expression vector of Claim 1; and

transfecting mammalian cells with the expression vector in order to express protein from the transgene.

11. The method according to Claim 10, wherein the mammalian expression vector is selected from a group consisting of pPGM-1 (KCCM 10232), pPGM-2 (KCCM 10338), and pPGM-3 (KCCM 10339).

FIG. 1

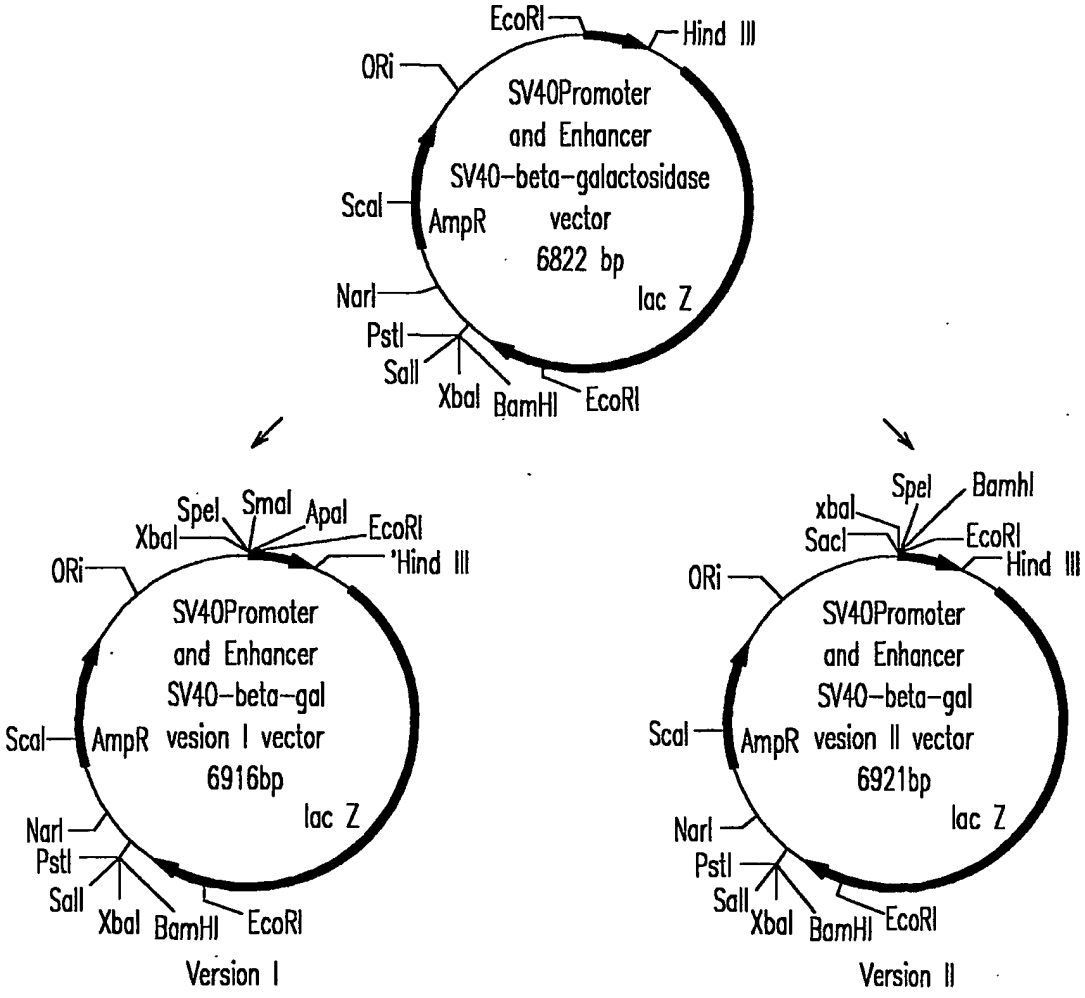
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FIG. 2

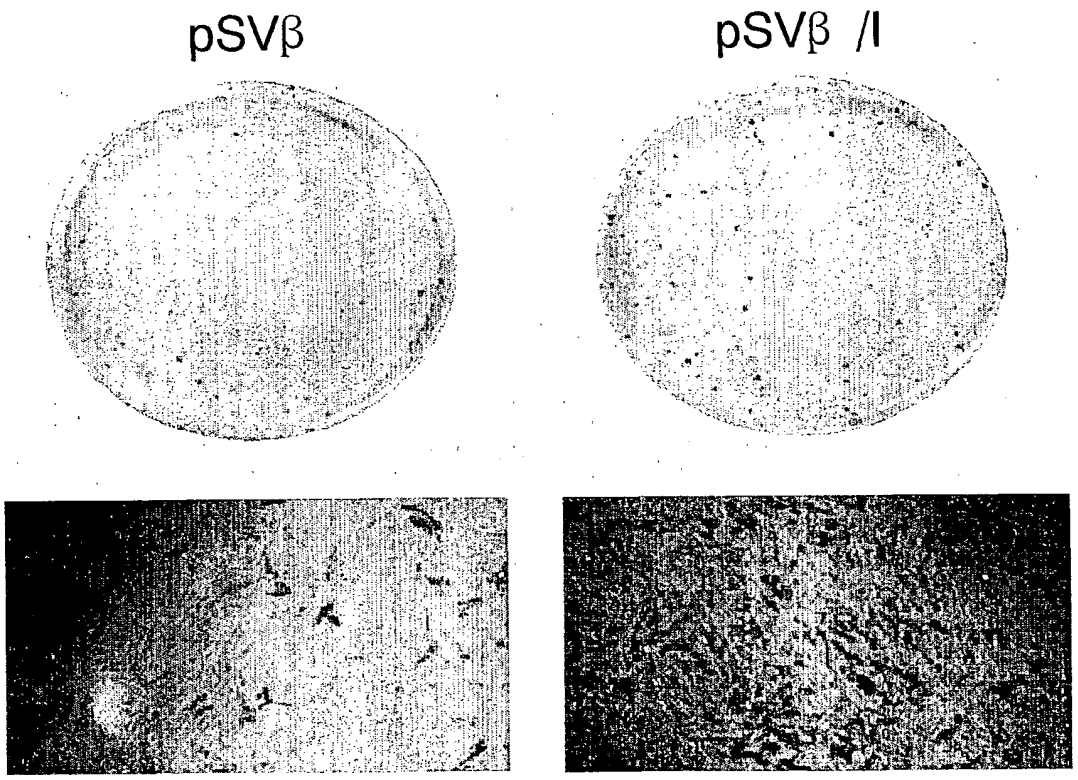
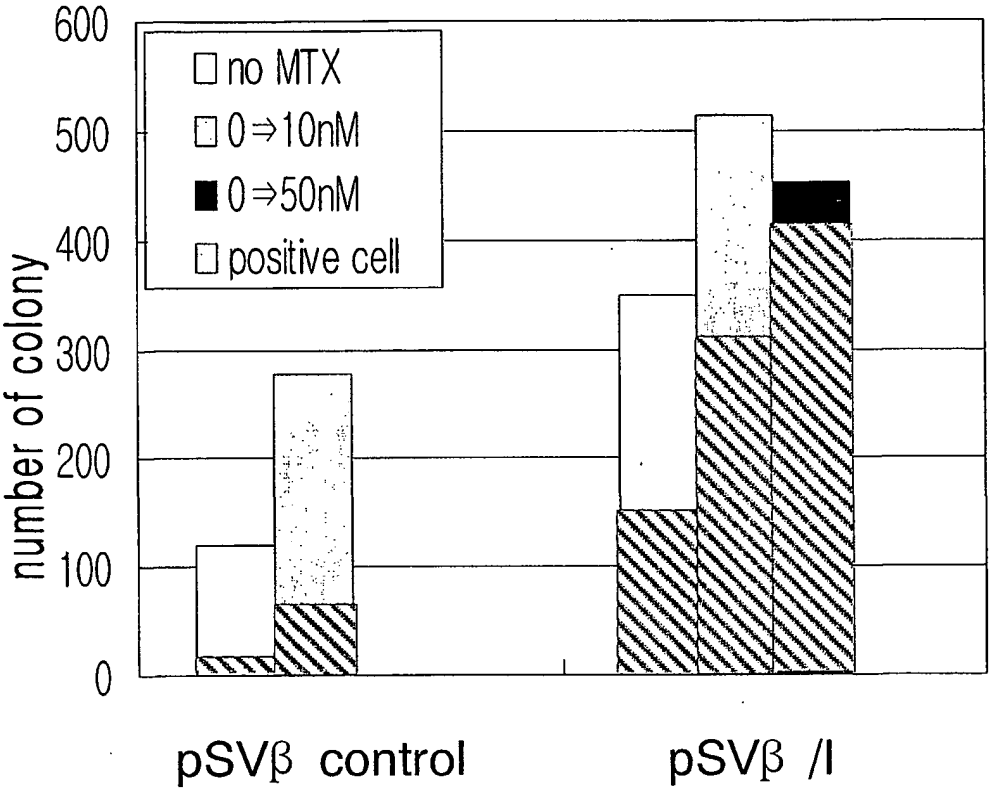


FIG. 3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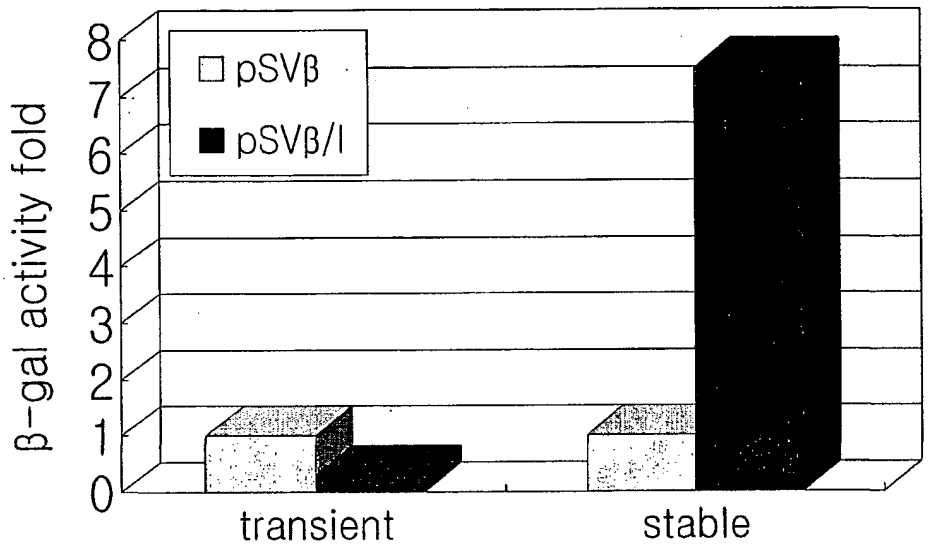
pSVβ /I

FIG. 4



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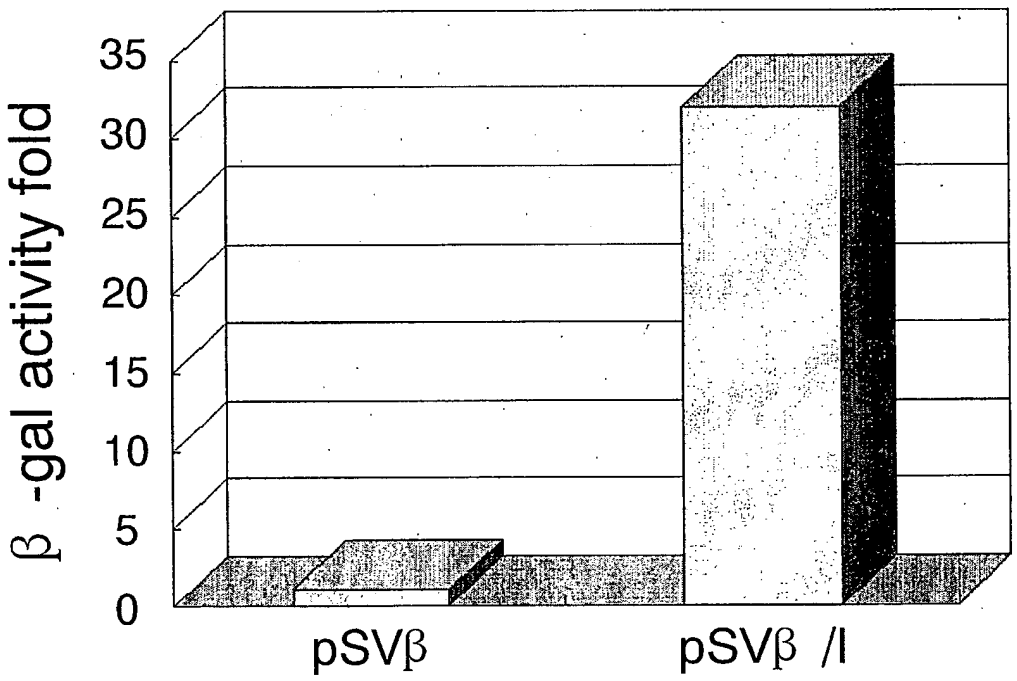

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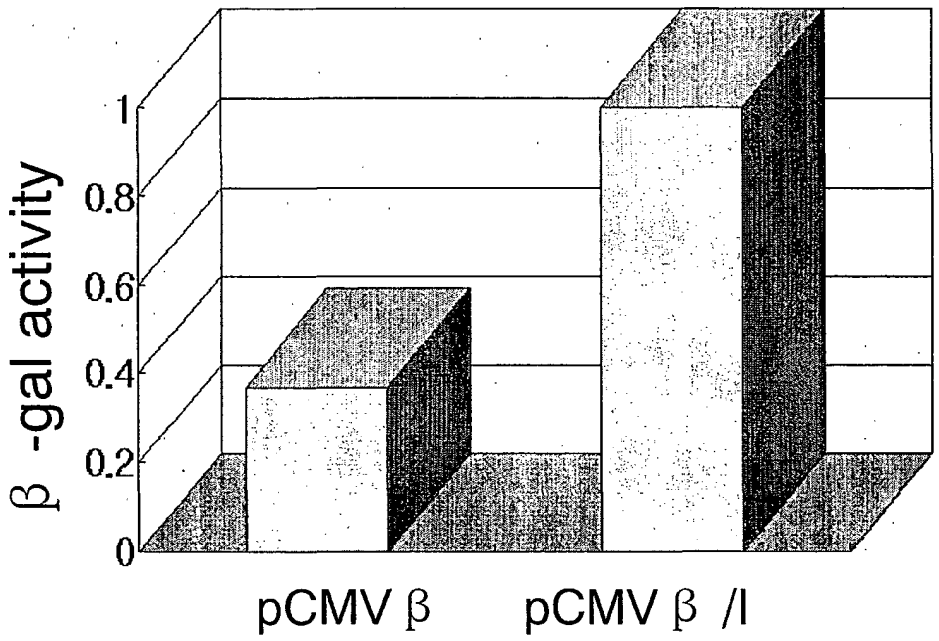

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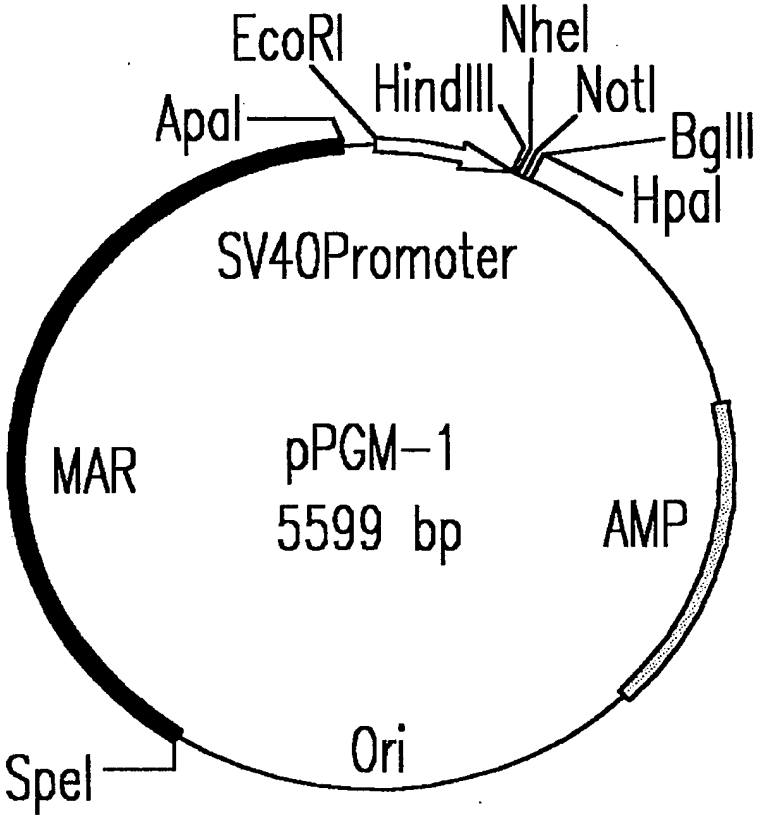

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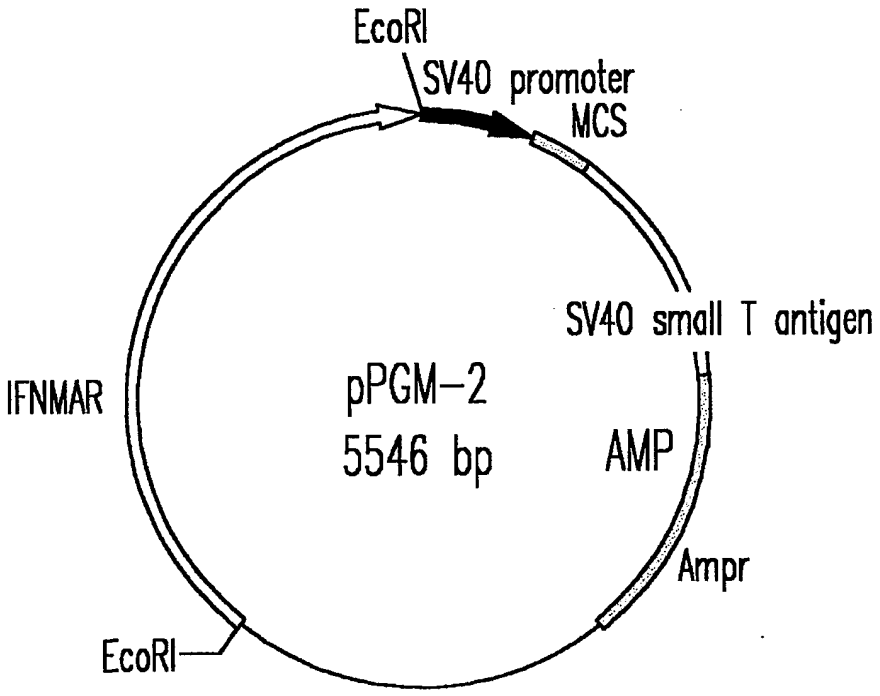
FIG. 8



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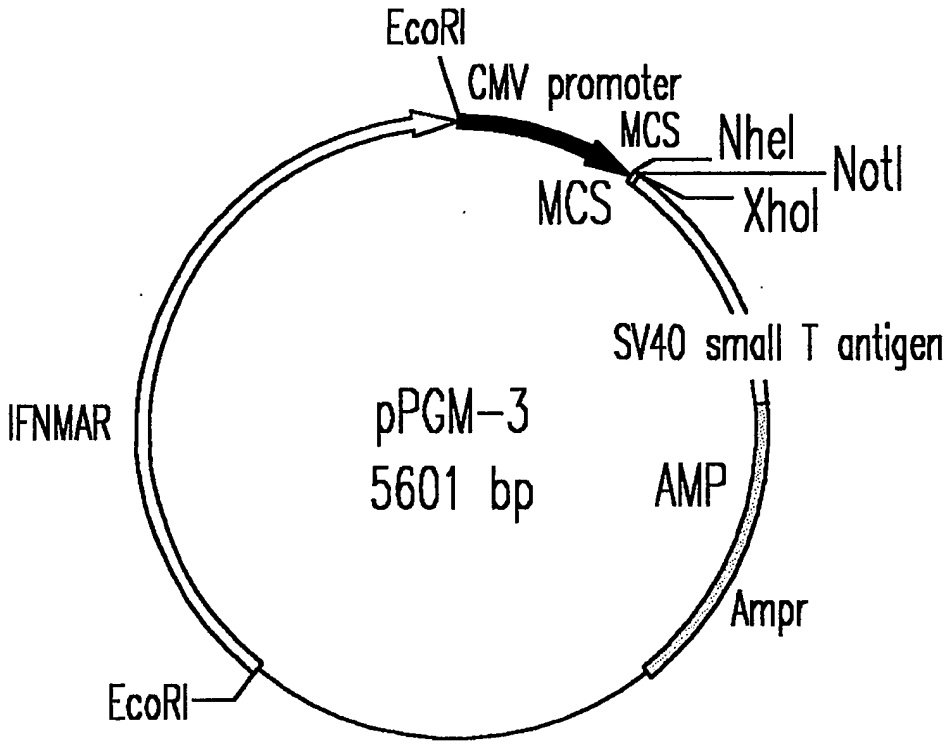
BamH I — BstX I — EcoR V

CAGTG GCGGC CGCTC GAGTC TAGAG GGCCC GTTTA AACAC GCGT. . . 3'

BstX I — Not I — Xho I — Xba I — Apa I — Pme I — Mlu I

FIG. 9



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*Nhe*I *Not*I *Xho*I

FIG. 10

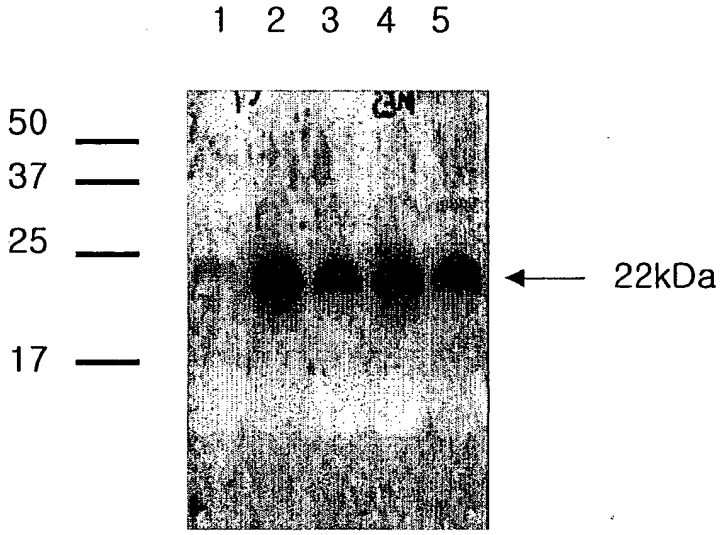
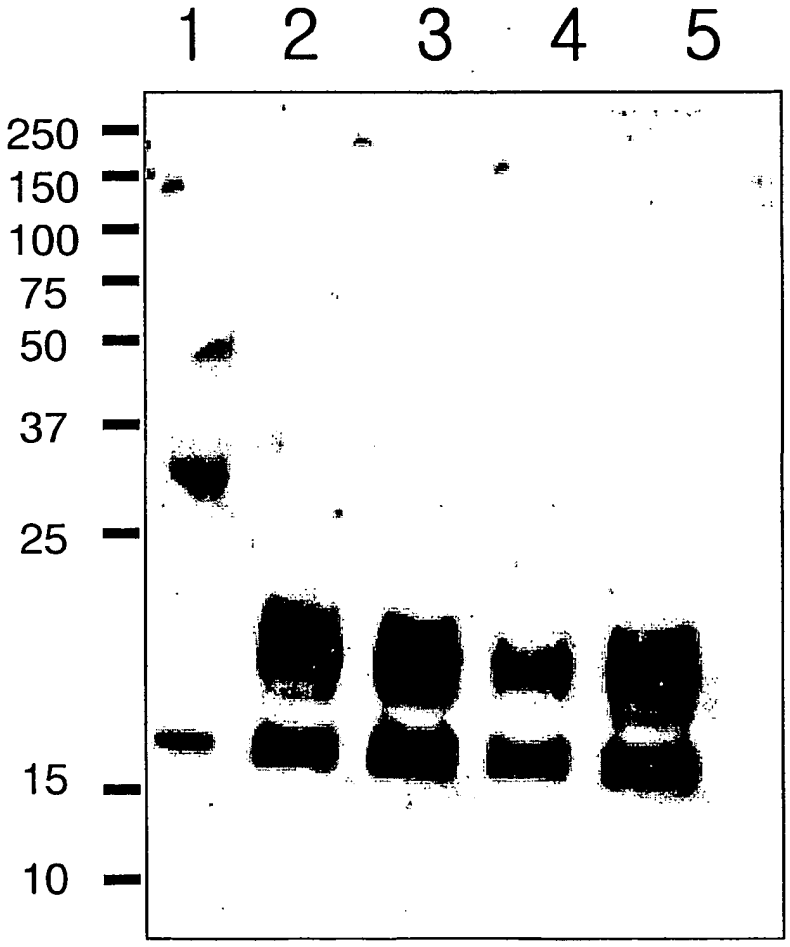


FIG. 11



Sequence Listing

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/02178

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7 C12N 15/85

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NCBI pubmed, KIPO NPS database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J Virol 1998 May;72(5):3720-8	1-5, 6-9, 10-11
X	J Virol 2000 Mar;74(6):2671-8	1-5, 6-9, 10-11
A	Hum Gene Ther 1999 May 20;10(8):1389-99	1-5, 6-9, 10-11
A	Acta Biochim Pol 1995;42(2):171-6	1-5, 6-9, 10-11
A	Biochemistry 1991 Feb 5;30(5):1264-70	1-5, 6-9, 10-11
A	Biochemistry 1990 Aug 14;29(32):7475-85	1-5, 6-9, 10-11

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 APRIL 2002 (24.04.2002)	25 APRIL 2002 (25.04.2002)

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Form PCT/ISA/210 (second sheet) (July 1998)